

FIG. 1. Sensitivity of the PCR assay. Shown are the results of PCR amplification of the serially diluted *L. denunciorum* (DD8) DNA analyzed on agarose gels. DNA was extracted from parasite cultures and amplified as described in Materials and Methods. Lane M, 1 kb Ladder (Gibco BRL); lane 1, 10 ng of DNA; lane 2, 1 ng of DNA; lane 3, 10 pg of DNA; lane 4, 1 pg of DNA; lane 5, 10 fg of DNA; lane 6, 1 fg of DNA.

Probe: Ld Ind kDNA

Human DNA:  $\xrightarrow{100 \text{ ng}}$

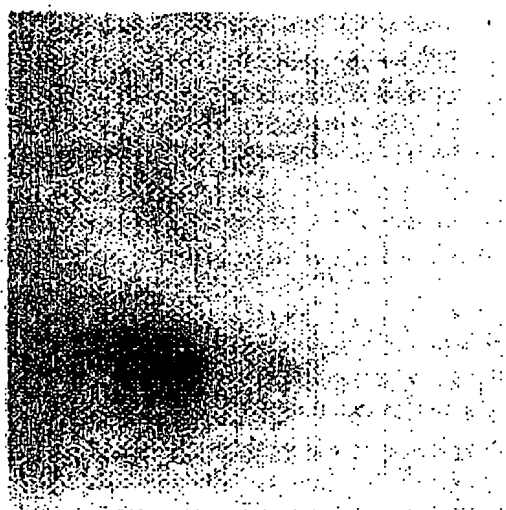
Primer Set: LdI1 & 2

Amt. Ld Ind DNA:  $\begin{matrix} 1 \text{ pg} \\ 0.1 \text{ pg} \\ 0.01 \text{ pg} \\ 0 \end{matrix}$

(Kb)

0.87 —

0.6 —



1 2 3 4

FIG. 2. Sensitivity of PCR amplification of *Leishmania* kDNA followed by Southern blot analysis. The PCR contained 100 ng of human genomic DNA and the indicated amount of total DNA from *L. donovani* DD8. The PCR product was probed with parasite kDNA and exposed for about 1 h. Lane 4 represents a PCR containing only human DNA as a control.

1 2 3 4 5 6 7 8 9 10 M 11 12 13

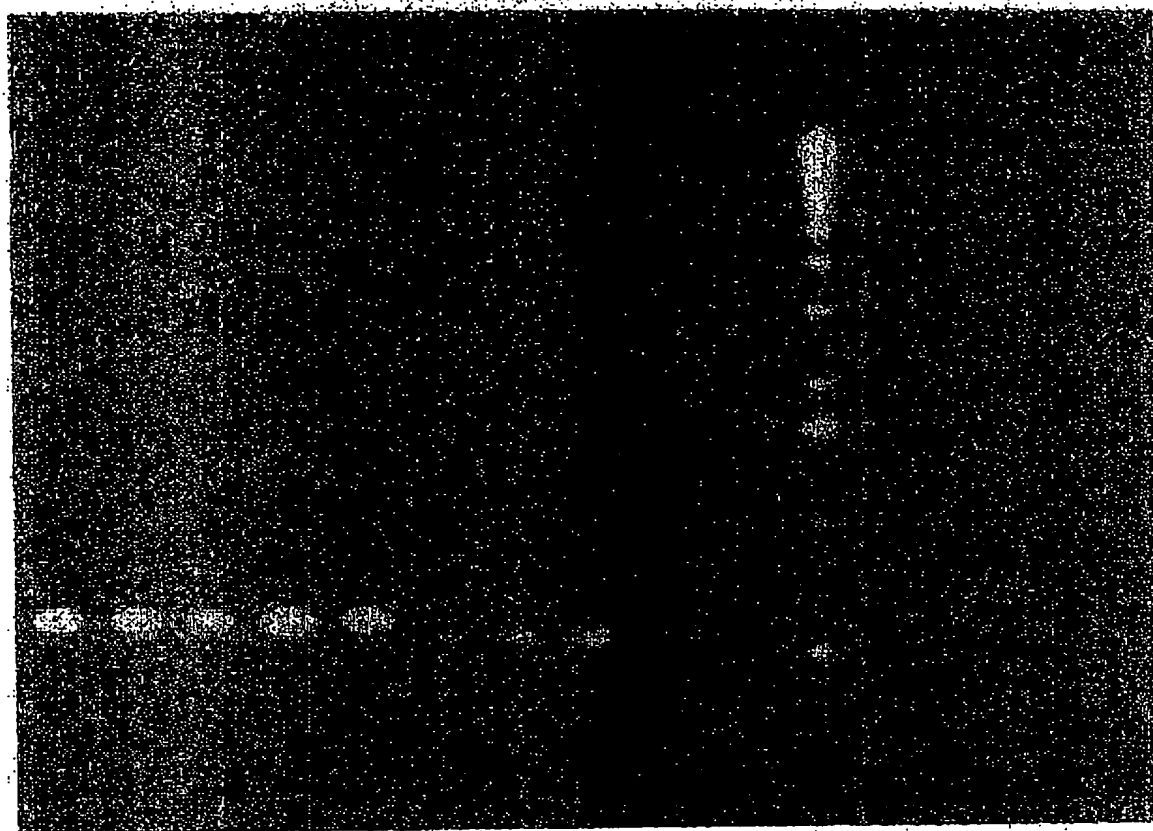
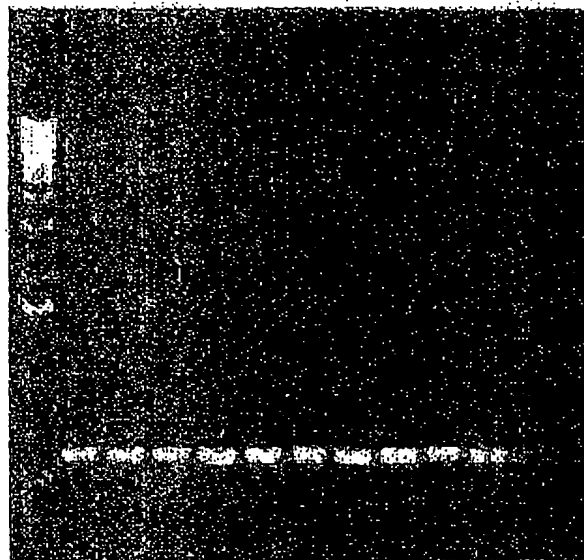


FIG. 3. Amplification of parasite DNA from various strains and isolates of *Leishmania*. DNA (1 ng) isolated from parasite cultures was subjected to PCR and analyzed. Lane 1, *L. donovani* AG83; lane 2, *L. donovani* DD8; lane 3, *L. donovani* IICB8; lane 4, *L. donovani* CB6; lane 5, *L. donovani* IICB 7 (PKDL origin); lane 6, *L. donovani* S; lane 7, *L. donovani* WR684; lane 8 *L. donovani* infantum; lane 9, *tropica* WR683; lane 10, *L. major* LV 39, lane M, 1-kb ladder, lane 11, *Plasmodium*; lane 12, *M. leprae*; lane 13, *M. tuberculosis*.

M 1 2 3 4 5 6 7 8 9 10 11



— 600 bp

FIG. 4. DNA amplification from recent field isolates of KA and KDL. DNA (1 ng) extracted from cultures of parasite isolates was used for PCR amplification. Lanes: M, 1-kb ladder; 1, KA-1; 2, KA-2; 3, KA-3; 4, KA-4; 5, KA-5; 6, PK-1; 7, PK-2; 8, PK-3; 9, PK-4; 10, PK-5; 11, isolate from a patient with cutaneous leishmaniasis.

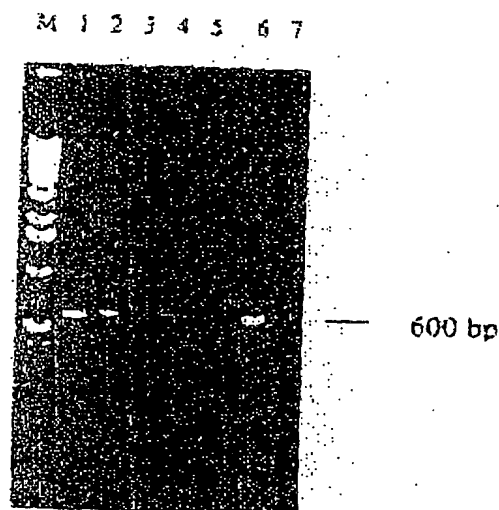


FIG. 5. PCR assay with clinical samples of KA and PKDL. DNA (100 ng) isolated from clinical samples was used for PCR amplification. Lane M, 1-kb ladder; lane 1, KA (bone marrow); lane 2, KA (blood); lane 3, malaria (blood); lane 4, tuberculosis (blood); lane 5, control from the area of endemicity (blood); lane 6, PKDL (skin lesion); lane 7, leprosy (lesion).

Fig 6. Sequence of PCR products with DNA isolated from *L. donovani* DD8 strain, isolates and clinical samples of KA and PKDL.

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1  gaattcgccg aaaaatgacc gaaaatgggc aaaaaaccca aacttttctg gtccrccggg
61  taggggcggt ctgcgaaaac cgaaaaatgg gtgcagaaat cccgttcaaa aaatagccca
121 aaatgccaaa aatcggtctc gagggcggaa actggggggt ggtgtaaaat aggggtcggt
181 ggaggggaaa ctgggggctc ggacgtgtgt ggatatggcc tgggtgggga ctttgagtg
241 ggttgtacct gtatgggggt tggacctgg cttgggggtt gggggttggg gtgggaaagg
301 ggtcggcgct atttggagtg acgttggctc ttttgataat tgatatctgc tctaaactgg
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661 ctatgaactt actagacata atttgtattt gatgctatag tgctactgat agagtgcacc
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781 gaagagacac cg

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